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Longitudinal clinico-serological analysis of anti-nucleocapsid and anti-receptor binding domain of spike protein antibodies against SARS-CoV-2

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ABSTRACT

Objectives: Monitoring the antibody responses to SARS-CoV-2 infection and its correlation to clinical spectrum of disease is critical in understanding the disease progression and protection against re-infection. We assessed the nucleocapsid (N) and receptor-binding-domain of spike (SRBD) protein specific IgG and neutralizing antibody (NAb) responses in COVID-19 patients up to 8 months and its correlation with diverse disease spectrum.

Methods: During the first wave of the SARS-CoV-2 pandemic, from 284 COVID-19 patients, 608 samples were collected up to 8 months post infection. The patients were categorized as asymptomatic, symptomatic and severe. The N and SRBD IgG and NAb titers were evaluated and correlated with clinical data.

Results: A steep increase in antigen specific antibody titers was observed till 40 days post onset of the disease (POD), followed by a partial decline till 240 days. Severe disease was associated with a stronger SRBD IgG response and higher NAb titers. The persistence of antibody response was observed in 76% against N, 80% against SRBD and 80% for NAbs of cases up to 8 months POD.

Conclusion: RBD and N protein specific IgG persisted till 240 days POD which correlated with NAb response, irrespective of individual's symptomatic status indicating overall robust protection against re-infection.

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Introduction

With over 221 million people infected across the globe, the pandemic of COVID -19 is still a public health emergency and posing a significant threat to life. After a period of initial global decline in COVID-19 cases, the virus has strongly re-emerged in many countries. During the first wave of SARS-CoV-2 infection in

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India, the maximum number of cases per day reached its peak during the months of September and October 2020, and subsequently declined until February 2021 followed by an upsurge (second wave) of the COVID-19 cases in May 2021 (WHO-COVID-19-global-data 2021).

The clinical manifestations due to SARS-CoV-2 infection can vary from asymptomatic to mild infection to severe acute respiratory distress syndrome (ARDS) (Singhal, 2020). Research indicated that about 40-45% of the SARS-CoV-2 cases are asymptomatic(Oran and Topol, 2020) and about 10% present with symptoms of severe disease such as increased respiratory rate, dyspnoea and low blood oxygen saturation (Brochot et al., 2020). These varied clinical manifestations can be evaluated using various biochemical markers (Ciaccio and Agnello, 2020)(Pourbagheri-Sigaroodi et al., 2020).

The approved method for diagnosis of the infection is real time RT-PCR (Definitions, 2020) and the reported duration for the RT-PCR positivity is from 3 days prior to onset of symptoms up to 83 days post onset of disease (POD)(Walsh et al., 2020). Since the viral detection period is so varied, understanding immune response is crucial.

Antibody response is one of the key factors for development of immunity and preventing re-infection. In our earlier study, we observed that the antibodies appear as early as the 4th day POD (Deshpande et al., 2020). However, the response to specific antigen may differ, due to the level of expression and immunogenicity and time. SARS-CoV-2 has four major structural proteins - spike (S), membrane (M), envelop (E) and nucleocapsid (N) (Kontou et al., 2020). Of the four structural proteins, N and S proteins are the primary viral antigens responsible for eliciting antibody response (To et al., 2020). In India, a vaccination drive was started on 16th January and as the waning of antibody is of major concern, it is pertinent to investigate the persistence of antibody response against COVID-19 virus (Brochot et al., 2020)(Choe et al., 2021a)(Choe et al., 2021b)(Dobaño et al., 2021)(Thangaraj et al., 2021).

Also, limited information regarding a combined analysis of SARS-CoV-2 antigen specific antibodies and neutralizing antibody responses over a longer period of time, its correlation with clinical findings and disease severity is hindering our understanding of the roles of humoral immunity in COVID-19 protection. Furthermore, the kinetics of antibodies against SARS-CoV-2 is of great importance after the introduction of the new vaccines as it will be useful in development of therapeutic and preventive modalities for halting the pandemic.

Here, we report immune responses of COVID-19 patients against N and SRBD proteins up to eight months POD. Also the levels of N and SRBD specific IgG were correlated with the plaque reduction neutralization (PRN) assay, hemoglobin (Hb), total Red blood cell (RBC) count, total white blood cell (WBC) count and platelet count in asymptomatic, symptomatic and severe symptomatic patients.

Methods

Ethical Statement

The study was approved by Institutional Ethics Committee of ICMR – National Institute of Virology.

Patients and Samples

In this study, a total of 608 serum/plasma samples were collected from 284 COVID-19 patients between April 2020 to February 2021 during the first wave of SARS-CoV-2 from designated COVID-19 hospitals in Pune Municipal Corporation (PMC) and Pimpri-Chinchwad Municipal Corporation (PCMC), Maharashtra, India. RT-PCR done from the throat/ nasal swabs collected from a subset of these patients revealed that the circulating SARS-CoV-2 strains were from the G (D614G) clade (Potdar et al., 2020)(Potdar et al., 2021). After the discharge of the patients, follow up blood samples were collected till a maximum period of eight months from the onset date of illness at PMC/PCMC clinic by the trained staff. None of the recovered patients were re-infected within the 8-month period of follow up. Among qRT-PCR confirmed positive cases, the participants were classified into three categories based on their clinical symptoms. Asymptomatic cases were patients who did not develop any symptoms throughout the course of the disease. Symptomatic cases were patients with fever, fatigue, body ache, diarrhoea, abdominal pain, dyspnoea and respiratory symptoms like runny nose, cough, sore throat, and nasal discharge, whereas patients with any of the following 3 criteria with or without the above symptoms were included in severe cases: Respiratory distress with breathing difficulty ($\geq$30 breaths/min) or Oxygen saturation with $\leq$90% at rest or chest imaging with $>$50% obvious lesions (Ministry of Health and Family Welfare., 2020)(Xiang et al., 2020). The study included 208 symptomatic, 60 asymptomatic and 16 severe symptomatic patients. Of the 284 patients, 132 patients provided multiple samples (56 patients provided 2, 23 patients provided 3, 19 patients provided 4, 21 patients provided 5, 3 patients provided 6, 6 patients provided 7, 3 patients provided 8 and 1 patient provided 10 samples) while 152 patients provided one sample. Case histories of all the patients were documented from hospitals. Hematological parameters like Hb, platelet count, total RBC count, total and differential WBC counts were recorded for 125 of the 284 patients.

SRBD protein specific SARS-CoV-2 IgG capture ELISA

Anti-SARS-CoV-2 SRBD protein IgG in human serum specimens was tested by coating the recombinant SRBD protein on to the microtitre wells followed by post-coating procedures. Serum samples were diluted in the ratio of 1:50 with sample diluent. Fifty microlitres each of the diluted samples, positive and negative controls were added to respective wells. ELISA plate was incubated at 37°C for 1 hour followed by washing with wash buffer 5 times. Fifty microlitres of ready to use anti-human IgG HRP was added to each well and was incubated at 37°C for 30minutes. After washing, 100μL of liquid 3,3′,5,5′-Tetramethylbenzidine (TMB) substrate was added and incubated at room temperature in the dark for 10 minutes. The reaction was stopped by adding 100μL stop solution (1N H_2SO_4) after 10 minutes. The absorbance was measured at 450nm. If OD value of sample tested exceeds 0.2 and sample OD/ negative control OD (sample ratio) $>$2.7, the sample was considered positive (Supplementary Figure 1).

SARS-CoV-2 recombinant N protein IgG capture ELISA

As described for S-RBD ELISA, similar procedures and criteria were followed for dilution of the samples, testing protocol and interpretation for recombinant N protein ELISA (Supplementary Figure 1).

Plaque reduction neutralization test (PRNT)

Total 298 samples were tested for PRNT (subset of samples tested for N and SRBD protein specific IgG by ELISAs); performed as described elsewhere (Deshpande et al., 2020).

Statistics

Descriptive statistics were calculated for continuous variables, counts and percentages for categorical variables. Mann–Whitney

Table 1
Demographic and hematologic features of COVID-19 patients

Variables	Reference ranges	General Observations (N = 284)	Disease severity Severe Symptomatic (N = 16)	Symptomatic (N = 208)	Asymptomatic (N = 60)	p value
Age <50* years	NA	182 (64.08%)	1 (6.23%)	135 (64.90%)	46 (76.66%)	
Age >50* years	NA	81 (28.52%)	15 (93.75%)	54 (27.27%)	12 (20%)	
Males	NA	158 (53.7%)	11 (68.75%)	116 (55.76%)	31 (51.66%)	
Females	NA	126 (46.3%)	5 (31.25%)	92 (44.23%)	29 (46.33%)	
White blood cells ($\times 10^3$ L^{-1})	4.00 to 10.00	8.0	10.07	7.8	7.4	$p > 0.05$
Neutrophil	40-60 %	63.8#	78.4#	62.36#	60.85#	$p < 0.0001$
Lymphocyte	20-40%	30.7	15.66#	32.04	33.76	$p < 0.0001$
Eosinophil	1-4 %	2.3	2.53	2.29	2.14	$p > 0.05$
Monocyte	2-8%	3.2	3.3	3.2	3.2	$p > 0.05$
Basophils	0-1%	0.0	0.0	0.0	0.0	$p > 0.05$
Red blood cells ($\times 10^{12}$ L^{-1})	4.00 to 5.50	4.8	4.39	4.76	4.89	$p > 0.05$
Haemoglobin ($g.dL^{-1}$)	12 to 17.5 (limits of males and females)	13.4	12.5	13.4	13.5	$p > 0.05$
Platelet ($\times 10^3$ L^{-1})	100 to 300 ($\times 10^9$ L-1)	277.0	265.8	275.26	283.78	$p > 0.05$
Fever	Above 98.6°F	150 (66.96%)	13 (81.25%)	137 (65.87%)	NA	
Cold/ Cough/ sore throat/ nasal discharge	NA	163 (72.76%)	14 (87.50%)	149 (71.63%)	NA	
Breathing problems/ Dyspnea	NA	33 (14.73%)	16 (100%)	17 (8.17%)	NA	
Diarrhea	NA	10 (4.46%)	1 (6.25%)	9 (4.33%)	NA	
Body ache	NA	64 (28.57%)	10 (62.50%)	54 (25.96%)	NA	
Fatigue	NA	25 (11.16%)	13 (81.25%)	12 (5.77%)	NA	

* Age not recorded for 21 (7.39%) patients. Symptomatic data recorded for 224 patients, 60 asymptomatic patients excluded. NA – Not applicable.
# - $p < 0.0001$.

U-tests and Kruskal-Wallis test were performed to compare the differences between groups. Pearson's correlation was drawn to evaluate correlation between two methods. Geometric mean titers were calculated for ELISA ratios and NAb titers. The analysis was performed on GraphPad Prism 9. Cox proportional hazards was evaluated for the association between neutrophil to lymphocyte ratio and antibody responses and Receiver Operating Characteristic (ROC) curves for SRBD and N IgG ELISAs against PRNT were plotted on IBM SPSS 26.

Results

Demographics and Clinical findings

For total of 284 patients, the median age of the study population was 38 years with interquartile range (IQR) of 26–51, of which (158) 55.6% of them were males and (126) 44.3% were females. About 21.12% and 78.88% patients were asymptomatic and symptomatic with mild to severe symptoms respectively. Of the 224 symptomatic patients, 7.14% patients presented with severe symptoms. Majority of the older males (62.50%) showed severe symptoms. All the hematological parameters were recorded for 125 patients. Further analyses revealed that patients with severe disease condition had higher neutrophil (78.4%) and lower lymphocyte counts (15.4%). The most common symptoms observed were cold/cough/sore throat/nasal discharge (72.76%), fever (66.96%), body ache (28.57%) and breathing problems / Dyspnoea (14.73%), while comparatively less common were fatigue (11.16%) and diarrhoea (4.55%)(Table 1).

The SARS-CoV-2 N and SRBD protein-specific IgG antibody response (n = 608) and NAb activities (n = 298) were investigated and correlated in acute (0-21 days POD) and convalescent (post 21 days POD) samples. A marked increase in the mean antibody titers from acute to convalescent samples was observed for both the protein specific IgG antibodies as well as for NAbs (Figures 1A, 1B, 1C). We observed SARS-CoV-2 SRBD specific IgG ELISA seropositivity in the majority of (78.94%) of COVID-19 samples (185/291 acute, 295/317 convalescent) (Figure 1A). However, N specific IgG antibody levels were detected relatively lower (74.83%) in all the samples (170/291 acute, 285/317 convalescent) (Figure 1B) compared to SRBD IgG antibody levels.

Additionally, SARS-CoV-2 NAb assessment data indicated the neutralizing activity among 75.83% COVID-19 samples tested (66/100 acute, 160/198 convalescent) (Figure 1C). Quantitatively, SRBD IgG levels showed positive correlation with N IgG levels ($r = 0.6848$, $p < 0.0001$) (Figure 1D). The SARS-CoV-2 NAb titers correlated more with SRBD IgG levels ($r = 0.7911$, $p < 0.0001$) (Figure 1F) than with respective N IgG levels. ($r = 0.7207$, $p < 0.0001$) (Figure 1E).

We examined and compared the IgG antibody levels against SRBD, N protein and the NAb titers at different time points post virus detection (Figure 2). From days 7 POD, there was a sharp rise in the average N and SRBD antibody ratios which continued to rise until day 40 after the onset of symptoms. Further, SRBD and N IgG levels showed a gradual decline till day 120. Both the N and SRBD IgG levels remained relatively stable from 120 to 240 days POD ($p < 0.05$, p = ns). Similar trend was observed for NAb response, with increase in average titers till 45 days POD and a decline till 120 days POD ($p < 0.05$, p = ns) (Supplementary Figure: 3). This was followed by a quiescent phase till 240 days POD. This persistence of immune response is of immense importance as it can provide an estimate of level of protection post natural infection.

Percent seropositivity was calculated for N IgG and SRBD IgG to observe the trends of antibody response in 284 patients till 240 days POD (Figure 3). During the early phases (0-7days) of infection, IgG antibodies were detected as early as day 3 and day 4 against SRBD protein and N protein of SARS CoV-2 respectively. During the first week POD, approximately one third of individuals showed ap-

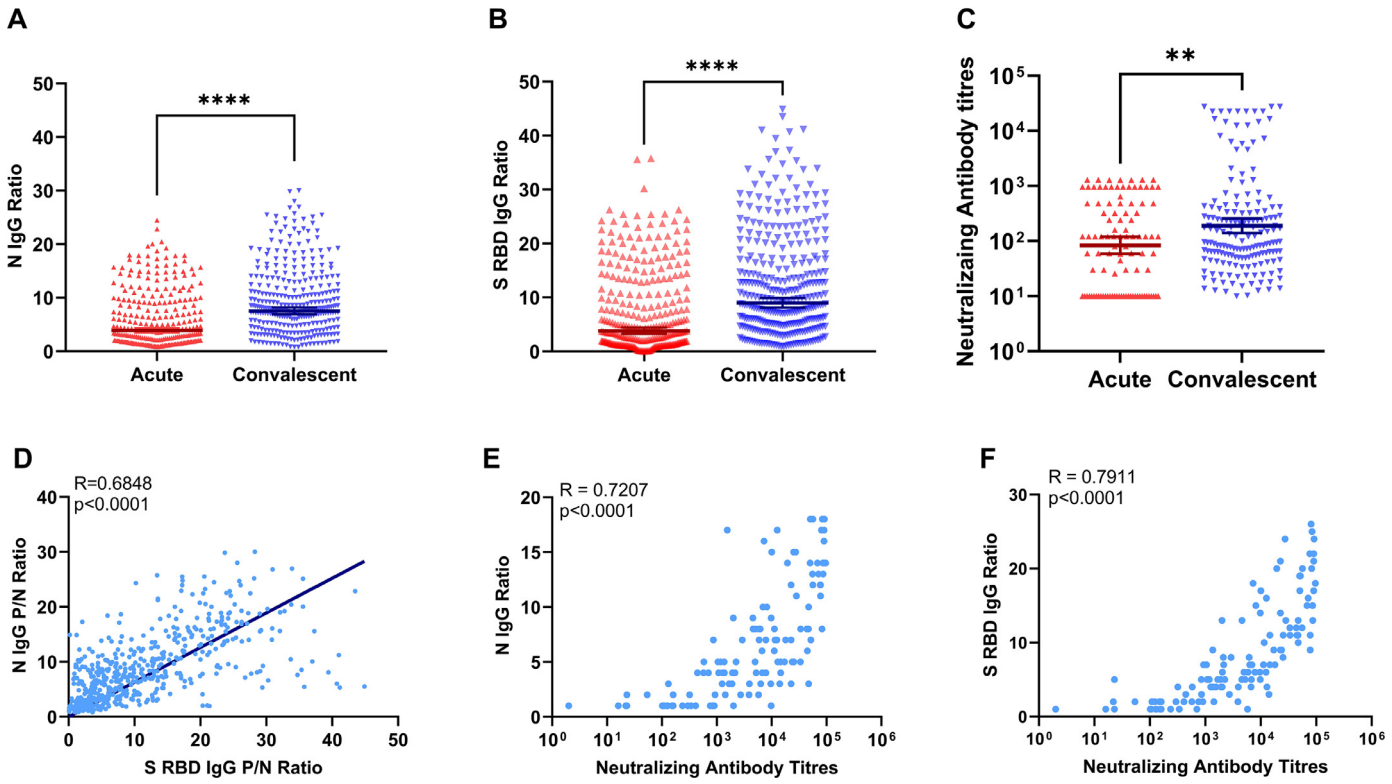

Figure 1. Humoral immune response assessed in 608 samples collected from 284 COVID-19 patients. Antibody responses to SARS-CoV-2 against N and SRBD proteins (A-B); neutralizing antibody activity (C) in acute and convalescent samples; Correlation of SRBD IgG with N IgG antibody levels (D) ($r = 0.6848$, $p < 0.0001$); Correlation of SARS-CoV-2 N IgG antibody levels and neutralizing antibody titers (E) ($r = 0.7207$, $p < 0.0001$); Correlation of SARS-CoV-2 SRBD IgG antibody levels and neutralization antibody titers (F) ($r = 0.7911$, $p < 0.0001$).

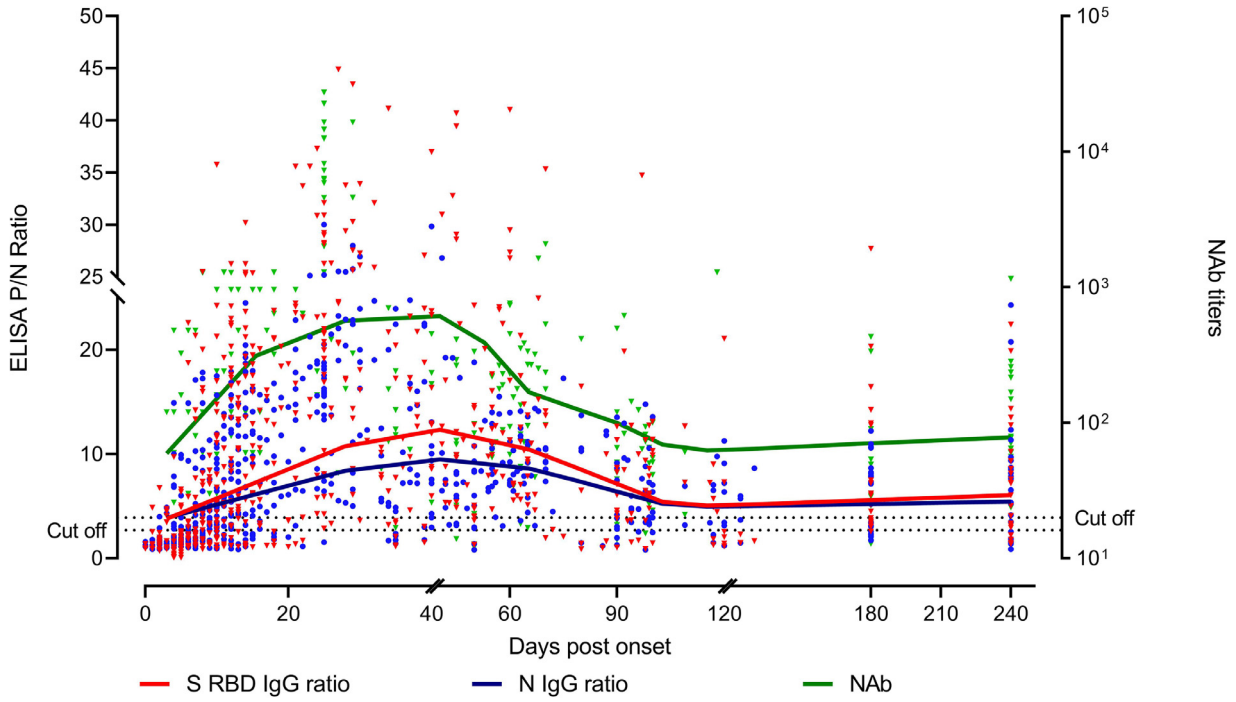

Figure 2. Longitudinal responses to SARS-CoV-2 IgG and NAbs till 240 days POD. The longitudinal trends of N (blue) and SRBD (red) specific IgG and neutralizing antibodies (green) with trend line showing geometric mean titers of each parameter for 608 samples from 284 patients collected over a period of 240 days POD.

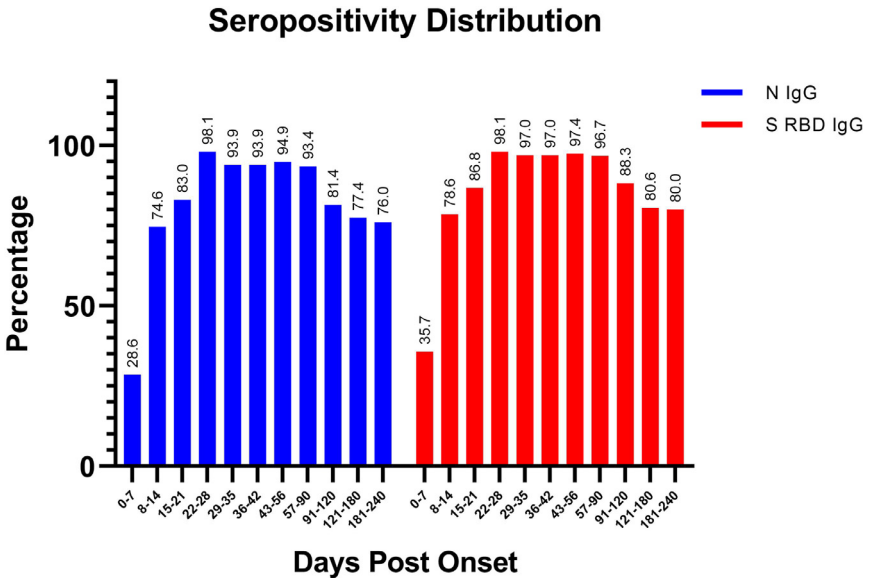

Figure 3. Seropositivity observed for N IgG (blue) and SRBD IgG (red) among 284 patients collected from 0-240 days POD. Seropositivity for both antibodies show a steep upward incline till 28 days POD and a gradual decline as the disease progressed.

pearance of IgG antibodies against SRBD protein (35.71%) versus N protein (28.57%) of SARS-CoV-2. Thereafter, the seropositivity rate for both the antigens increased simultaneously until week 4 and reached 98.07% for antibodies detected against SRBD and N proteins. From week 5 up to 12 weeks the positivity remained relatively constant (93.44 - 97.73%) and this period denoted the timeframe of maximum seropositivity for antibodies against N and RBD proteins. At the beginning of week 13 to end of week 17, the N protein IgG levels substantially dropped to 81.39% and that of RBD declined slightly to 88.37%. From week 18-27 both SRBD and N protein specific seropositivity stagnated to 80.64% and 77.41%.The seropositivity remained near constant for both antibodies till week 35.

Among 284 patients, the average IgG concentration (P/N ratio) against SRBD and N among symptomatic cases (SRBD ratio = 10.30; N ratio = 7.69) and asymptomatic cases (SRBD ratio = 10.02; N ratio = 8.66) was not significant (Figure 4). The average IgG ratio in severe symptomatic cases for N antigen (N ratio = 6.98) showed comparable, albeit slightly lower, ratio as compared to the symptomatic and asymptomatic cases, but showed higher values ($p < 0.05$) of average SRBD IgG ratio (SRBD ratio = 16.79). The average RBD and N IgG ratios showed very little variation among male (SRBD ratio = 10.90; N ratio = 8.08) and female (SRBD ratio = 10.06; N ratio = 7.89) patients as well (data not shown). However there was a slight variance between the protein-specific IgG immune response mounted when comparing individuals on either side of age 50 years. Patients above the age of 50 years (SRBD ratio = 11.97; N ratio = 8.63) showed a slightly higher antibody levels as compared to those below the age of 50 years (SRBD ratio = 9.56; N ratio = 7.32).

Considering neutrophil to lymphocyte ratio (NLR) is linked to innate immunity (Zhang et al., 2020) and is an early warning signal of severe COVID-19 (Xia et al., 2020), we analyzed NLR data against IgG response among asymptomatic, symptomatic and severe symptomatic patients (n = 125). Both N and SRBD IgG ratios were high with increased NLR in severe symptomatic patients (Figure 5A, 5B). Some symptomatic patients exhibited high NLR but the cumulative NLR of this category was within range. Also, the IgG ratios for both N and SRBD for symptomatic patients were not associated with increase in the NLR.

We then performed a time-dependent covariate Cox regression analysis of antibody responses (adjusted for sex and stratified for NLR at the time of sampling) on subsequent sampling (Figure 6). The development of SARS-CoV-2 RBD IgG antibodies was positively associated with high NLR in regression analysis with a hazard ratio (HR) for time of last sampling 1.061 ($p < 0.001$). The responses to the N protein (HR = 0.931) were not linked to high hazard ratio.

The levels of N and SRBD specific IgG antibody were also evaluated for correlations with all the hematological parameters in asymptomatic, symptomatic and severe symptomatic patients. With increase in POD, the increase of N and SRBD IgG positively correlated with the increase in WBC and platelet counts in severe patients, the correlation coefficient (r) was 0.53 and 0.38 for N and 0.52 and 0.39 for SRBD IgG respectively. Negative correlation was observed between RBC counts and N (r = -0.47) as well as with RBD (r = -0.50). However, no correlation with changes in Hb was observed in severe patients. The changes in N and SRBD specific IgG antibodies showed no significant correlations with any of the hematological parameters in asymptomatic and symptomatic patients (Supplementary Figure: 2).

Discussion

In SARS-CoV-2 infection, waning of immunity and probability of re-infection is a major concern and there are reports affirming that antibody titers decline more quickly in asymptomatic or mild symptomatic cases than severe cases (Yamayoshi et al., 2021). Studies showing longevity of antibody response in SARS CoV-2 in a large sample size are very scarce with contrasting results (Hartley et al., 2020)(Choe et al., 2021b). Here, we provide a comprehensive analysis of antibody dynamics and persistence of antibody response by evaluating protein specific IgG levels in 284 individuals with varied disease severity up to 240 days POD.

The antibodies against SRBD IgG appeared slightly earlier and remained substantially more persistent than the N protein specific IgG throughout the course of the study (240 POD). Notably, the antibody levels tend to decrease with increased interval between days post onset of symptoms until they reached a constant value. Although seropositivity reaches its maximum by week 4, average relative IgG titers against both N and SRBD antigens continue to increase till week 6 followed by a steady decline in average relative titers during weeks 7-17. After this point, the average titers of anti-SRBD & anti-N IgG antibodies remained constant till week 35.

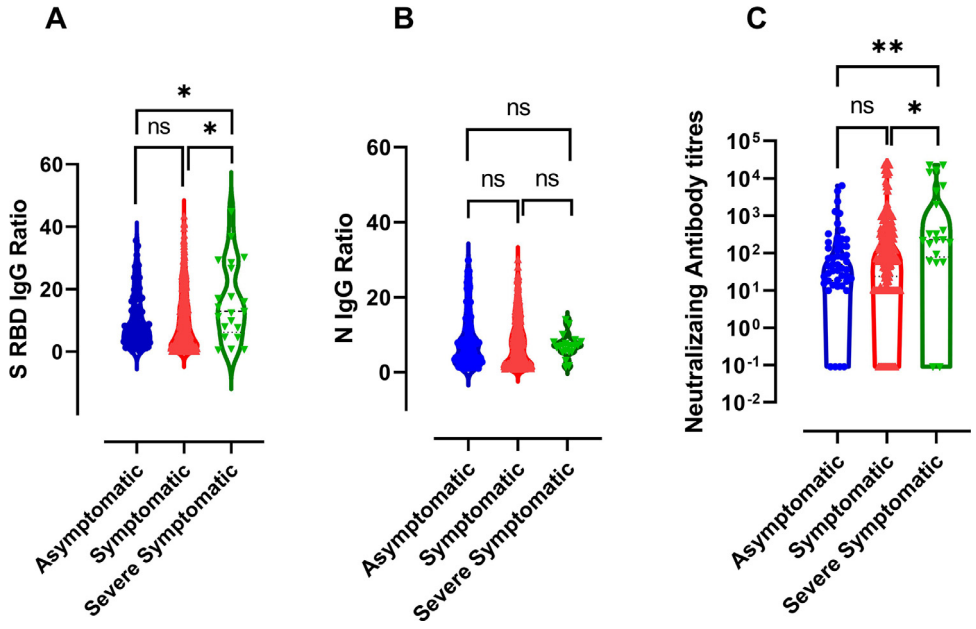

Figure 4. Levels of IgG antibodies and titers of NAbs against SARS-CoV-2 of asymptomatic (blue), symptomatic (red) and severe symptomatic (green) cases among 284 patients collected from 0-240 days POD. Antibody levels are expressed as ratio values (A, B) and NAb titers are plotted (C). The median and quartiles were represented in the violin plots. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$)

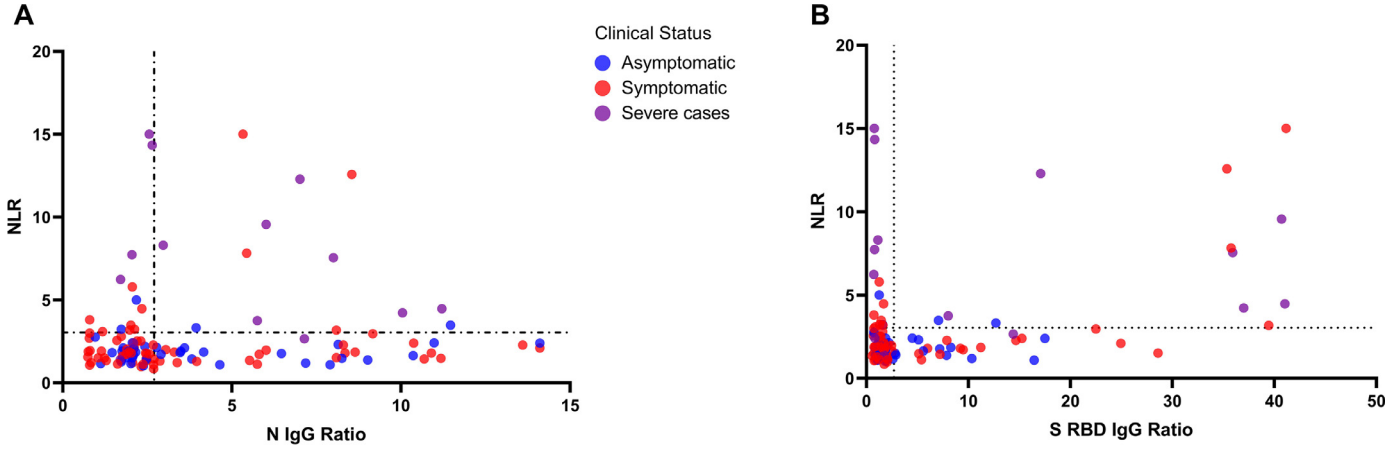

Figure 5. Immune response across diverse disease severity with respect to neutrophil to lymphocyte ratio (NLR) and IgG antibody levels (N = 125). Association between A) N IgG ratio and NLR (cut-off = 3.04); B) SRBD IgG ratio and neutrophil to lymphocyte ratio for 125 patents. (Asymptomatic = 42, Symptomatic = 68 and severe symptomatic = 15 cases.)

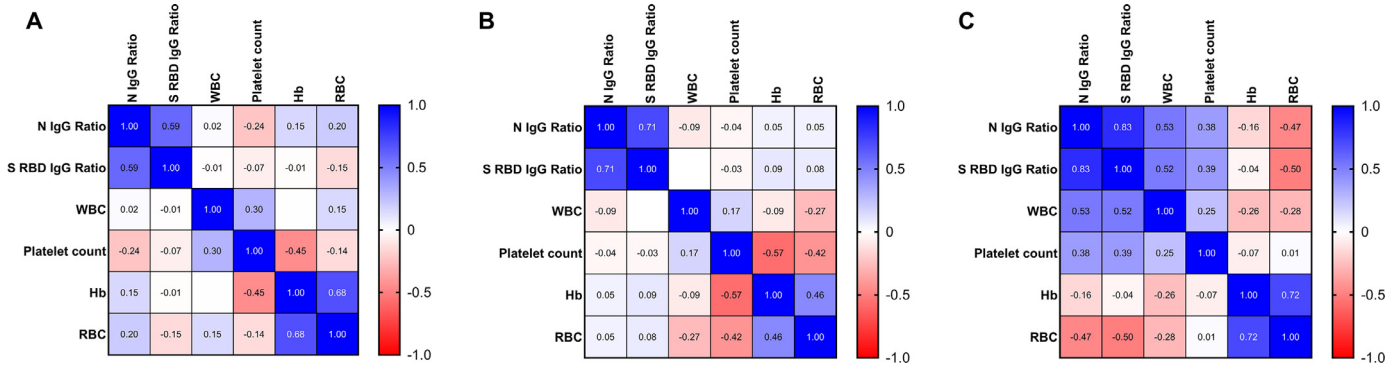

Figure 6. Associations of immune response and disease severity correlogram of COVID-19 patients: A) Asymptomatic cases, B) Symptomatic cases and C) Severe symptomatic cases). Spearman rank order correlation values (r) are shown from red (-1.0) to blue (1.0); r values are indicated by color and square size.

It is worth mentioning that various factors may predict an enhanced initial antibody response against SARS-CoV-2 and the persistence of antibodies over time (Terpos et al., 2021). Of the measured SARS-CoV-2 antibodies, the IgG response against the SRBD domain was associated with NAbs ($r = 0.79$) independent of other factors such as sex or age. This indicated that SRBD specific antibodies in the patient sera meant improved patient survival rate supporting the concept that these antibodies are a major contributor to the protective effect of humoral immunity in COVID-19. This finding may have implications in the anticipated protection against re-infection over time. Additionally, the SRBD specific IgG response showed comparatively higher average levels of antibody ratios in patients with severe disease, than the asymptomatic and symptomatic cases. A previous study reported that the severe disease was associated with more robust serological responses including early seroconversion (<day 16) and higher IgG levels (Zhang et al., 2020), which is in agreement with our study observation. Our findings in turn indicate higher titers of NAbs among severe cases. Conversely, the overall quantitative IgG response against N protein did not differ significantly based on the disease severity, demonstrating that it is independent of the severity of the disease. Differing from other studies (Hibino et al., 2021)(Long et al., 2020), we witnessed that there was no early waning in IgG titers in asymptomatic or symptomatic cases. This difference in observations could probably be attributed to the variable genetic makeup and individual overall immune status. Also the differences in detection methods and disease severity in different cohorts may be responsible for such variations (Bölke et al., 2020).

In addition, contradictory to some studies (Santis et al., 2020)(Marklund et al., 2020)(Lee et al., 2020), not all patients, irrespective of their symptomatic status, had developed an IgG response to SARS-CoV-2. Of the sequential samples received, 7 patients failed to mount a response against N protein, 1 failed to mount a response against SRBD protein, 5 patients failed to mount a NAb response, 2 failed to mount a response altogether against both antigens and 2 patients did not mount any IgG or NAb response at any given time point. These patients or a subset of these patients can be considered as 'non-responders' and further studies are needed for confirmation as these patients hold the key to complete disease elimination following the global vaccination drives.

Though the global studies show upwards of 45% COVID-19 cases as being asymptomatic, the number was much lower in our study due to the selection bias of hospitals to admit patients mainly based on their severity of symptoms. Based on clinical data, mild to severe symptoms were observed more among the older male population in the study. The recent report indicated that the NLR was identified as a powerful predictive and prognostic factor for severe COVID-19 and systematic inflammatory response (Liu et al., 2020)(Yang et al., 2020). Though the number of severe symptomatic cases was less compared to symptomatic cases, in our observations, more severe cases presented with high NLR and high IgG antibody levels. The changes in N-IgG and SRBD IgG antibody response showed no significant correlations with Hb, total RBC count, total WBC count and platelet count in asymptomatic and symptomatic patients. Furthermore, the high disease severity was associated with an increase in the neutrophils and a decrease of lymphocyte count. Our observations were corroborated by other studies as well and a decrease of lymphocyte count may be attributed to SARS-CoV-2 induced syncytia formation leading to lymphocyte loss in the patients with COVID-19 (Aschenbrenner et al., 2021).

The limitations of this study include unavailability of data on virus titers during SARS-CoV-2 infection which would have emphasized a better correlation between disease severity and immune response. Due to limited availability of clinical data, we could only analyze NLR but other immunological markers of cell mediated immunity also need to be studied in order to understand the diverse behaviour of immune responses. Nevertheless, this study tried to elucidate the understanding of varied antibody dynamics among infected patients along with disease severity and its clinical correlation with NLR which is very important clinical marker used for early screening of critical illness of patients. Current pandemic status necessitates the assessment of the antibody response for a prolonged period in COVID-19 patients to establish a link between the presence of antibodies and the level of protection against re-infection.

In conclusion, our study demonstrated the persistence of N and SRBD IgG response up to 8 months irrespective of the disease spectrum along with the strong longitudinal responses elicited against SRBD protein and correlated with the NAb, which in turn predicts the protective immunity. This data may help in vaccination strategies for those who were previously infected with COVID-19 and public health decisions.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2021.09.024.

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